

Kentucky
Agricultural Experiment Station

University of Kentucky

**THE IRON AND COPPER CONTENT OF EGG
YOLK**

BULLETIN NO. 342

(RESEARCH BULLETIN)



Lexington, Ky.

June, 1933

(133)

EXPERIMENT STATION STAFF

BOARD OF CONTROL

Richard C. Stoll, Chairman, Lexington, Ky.
E. B. Webb, Lexington, Ky.
R. G. Gordon, Louisville, Ky.
James Park, Lexington, Ky.
Joe B. Andrews, Covington, Ky.

Frank L. McVey, President

Thomas P. Cooper, Dean and Director

ADMINISTRATION

T. P. Cooper, Director
D. H. Peak, Business Agent
O. L. Ginocchio, Secretary

AGRONOMY

George Roberts, Head
E. J. Kinney, Associate Agronomist
P. E. Karraker, Asst. Agronomist
J. F. Freeman, Asst. Agronomist
W. D. Valleau, Plant Pathologist
E. N. Fergus, Asst. Agronomist
J. B. Kelley, Agricultural Engineer
E. M. Johnson, Asst. Plant Pathologist
C. E. Bortner, Asst. in Agronomy

FARM ECONOMICS

W. D. Nicholls, Head
W. L. Rouse, Farm Management
Z. L. Galloway, Farm Management
Merton Oyler, Rural Life Studies
George B. Byars, Farm Organization
Bruce Poundstone, Farm Management

FEED CONTROL

J. D. Turner, Head
H. D. Spears, Chemist
W. G. Terrell, Inspector
Fred Fitschen, Inspector
L. V. Amburgey, Microscopist

FERTILIZER CONTROL

H. E. Curtis, Head
Harry Allen, Chemist
Lelah Gault, Asst. Chemist
Robert Mathews, Inspector

HOME ECONOMICS

Statie Erikson, Head
Ruth Boyden, Assistant

HORTICULTURE

A. J. Olney, Head
C. S. Waltman, Assistant
E. M. Emmert, Assistant

ANIMAL HUSBANDRY GROUP

E. S. Good, Chairman
W. S. Anderson, Horses
E. J. Wilford, Swine, Meats
W. J. Harris, Beef Cattle
J. Holmes Martin, Poultry
Fordyce Ely, Dairy Husbandry
W. C. Eskew, Cream Grading
J. W. Nutter, Dairyman
Amanda Harms, Asst. Path. Bact.
G. D. Buckner, Animal Nutrition
W. M. Insko, Jr., Asst. Poultry Husb.
E. A. Baute, Poultry Improvement
Harold Barber, Head Herdsman

ANIMAL PATHOLOGY

W. W. Dimock, Head
Philip R. Edwards, Bacteriologist
F. E. Hull, Asst. Veterinarian
D. J. Healy, Bacteriologist

CHEMISTRY

J. S. McHargue, Head
S. D. Averitt, Chemist
O. M. Shedd, Chemist
W. R. Roy, Asst. Chemist
Robert K. Calfee, Asst. Chemist
David W. Young, Assistant

CREAMERY LICENSE SECTION

J. D. Foster, Inspector, in Charge
N. J. Howard, Inspector

ENTOMOLOGY AND BOTANY

W. A. Price, Head
Mary L. Didlake, Asst. Entomologist
H. H. Jewett, Research Asst. Entgst.
Jessie Taylor, Seed Analyst
C. O. Eddy, Assoc. Entomologist
E. C. Vaughn, Seed Improvement
Encl Deen, Seed Inspector

MARKETS AND RURAL FINANCE

H. B. Price, Head
Dana G. Card, Prices
Clifton J. Bradley, Rural Finance
C. D. Phillips, Markets
Olin M. Farrington, Markets

PUBLIC SERVICE LABORATORY

L. A. Brown, Head
A. L. Meader, Asst. Chemist
James H. Martin, Asst. Chemist
Geo. S. Terry, Bacteriologist
Harvey Cunov, Asst. Bacteriologist
Todd Green, Asst. Bacteriologist

ROBINSON SUBSTATION

(Quicksand, Ky.)

R. W. Jones, Superintendent
G. H. Wiggins, Forester

WESTERN KY. SUBSTATION

(Princeton, Ky.)

S. J. Lowry, Superintendent
L. M. Caldwell, Assistant

BULLETIN NO. 342

(RESEARCH BULLETIN)

The Iron and Copper Content of Egg Yolk

By S. E. ERIKSON, R. E. BOYDEN, J. HOLMES MARTIN and
W. M. INSKO, JR.

The importance of iron and of foods high in iron for hemoglobin building has been reviewed by Robscheit-Robbins (1). The egg yolk, as a food rich in iron, has been demonstrated to be of value in the regeneration of blood in hemorrhagic anemia by Whipple and Robscheit-Robbins (2). Gibson and Howard (3) recommend the use of egg yolk in pernicious anemia. Miller and Forbes (4), investigating the utilization of iron by the albino rat, found eggs of value as a source of iron.

The presence of copper in plant and animal tissues was noted more than a hundred years ago, but interest in it, from the nutrition standpoint, was not marked until its relationship with iron in hemoglobin formation was brought out in 1928 by Hart, Steenbock, Waddell and Elvehjem (5) and McHargue, Healy and Hill (6). Since then much stimulation has been given to investigations to determine the copper content of tissues and its specific function. From the evidence available, it is obvious that both iron and copper are important factors in nutrition because of their function in hematopoiesis. In the chick embryo, the building of hemoglobin starts very early. If the constituents for its formation should be deficient or lacking, with a consequent anemia, it would not be improbable to find the anemia responsible for some of the fairly high mortality of the embryos.

Payne (7) and Riddle (8) agree that avian embryo mortality shows two distinct high peaks, the first attaining its maximum

on the third and fourth days of incubation and the second on the final two or three days. Riddle suggests that the mortality of the first period may be due to failure of "respiratory adjustment". If even a slight lag in the formation of the blood and circulatory system should occur at this period, the result might be a less efficient adjustment to changing gas pressure and this in turn would be likely to result in death of the embryo.

Whether the levels of copper and iron values of eggs are subject to variations due to conditions of production is a matter of dispute. Sherman (9) thinks it is doubtful that the iron content of eggs can be increased by feeding iron-rich feed to poultry. Elvehjem, Kemmerer, Hart and Halpin (10) found that neither the iron nor the copper content of eggs was increased by adding, in the form of sulfate, 50 mg. of iron and 0.5 mg. of copper daily to the basal ration. Cunningham (11) tried adding to the diets of hens a very small amount of copper, 0.07 mg. to one lot and 0.27 mg. to another, per day, to note its effect on the storage of copper in the egg. He found no significant difference in the copper content of the eggs from the two groups and only a very slight increase in iron in the eggs from the group fed the larger amount of copper. Both groups, however, showed slightly lower values than those from a lot fed the normal stock rations without mineral addition.

Sherman, in the fourth edition of "Chemistry of Food and Nutrition" (9), gives the iron value of egg yolk as 0.003 percent. Peterson and Elvehjem (12) give the iron as 0.0076 percent in one sample of undried egg yolk.

Wide differences occur in values given for copper, which may be due to recent modifications and improvements in methods of analysis. Fleurent and Levi (13) found 20 mg. of copper per kilogram of dried egg yolk. Guerithault (14) gives 1.8 mg. per kilogram as the value for the fresh material. McHargue (15) found 3 mg. of copper per kilogram of fresh, or 6 mg. per kilogram of dry egg yolk. Lindow, Elvehjem and Peterson (16) found 8.0 mg. of copper per kilogram of dry, or 4.0 mg. per kilogram of fresh egg yolk. In the light of these investigations, the

problem presents itself as to whether variations occur in the content of copper and iron of egg yolk and, if so, the determination of the significance of the variations and the factors responsible for them.

PROCEDURE

The copper and iron problem is part of a larger study on some factors that may affect the nutritive quality of eggs.*

One hundred and fifty single-comb White Leghorn pullets of approximately the same age, size and sexual maturity were divided into 6 pens of 25 each and placed in houses of identical constructions and orientation. All the pullets were from high-laying parent stock in the Kentucky Experiment Station breeding flock. The pens were divided into 3 pairs, and one from each pair received cod liver oil in addition to the following all-mash ration which was fed *ad libitum* to all pens:

All-mash mixture fed dry in hoppers

Ground yellow corn	65.0 percent
Wheat bran	10.0 percent
Wheat middlings	10.0 percent
Meat scrap (50% protein)	7.5 percent
Dried buttermilk	2.5 percent
Finely ground limestone	2.0 percent
Steamed bone meal	2.0 percent
Salt	1.0 percent

Coarsely ground limestone (approximately 99% CaCO_3) and water were available in all pens at all times. The pens were treated as follows:

First Pair. Confined to house, no direct sunshine, only sunlight received thru common window glass.

Pen 1. No cod liver oil. Pen 2. Cod liver oil, 2 percent of the mash.

Second pair. Confined to house and wire-screened, wire-floored sun porch.

*See Kentucky, Bulletins 335 and 337 for other phases of the investigation.

Pen 3. No cod liver oil. Pen 4. Cod liver oil,
2 percent of the mash.

Third pair. Allowed range of bluegrass yard.

Pen 5. No cod liver oil. Pen 6. Cod liver oil,
2 percent of the mash.

All hens were trapnested and eggs were collected from six hens from each pen over a period of ten days for the determination of both copper and iron and from the same six hens over an additional ten-day period for iron analyses. All the eggs collected were analyzed. Hens were chosen whose previous egg production record showed an average of 5 or 6 eggs in 10 days.

Eggs were broken against the edge of a beaker and the white and yolk separated. Care was taken to see that the white was separated as completely as possible from the yolk. No metal was at any time allowed to come in contact with the egg contents.

A weighed portion (average 14 grams) of the yolk of each egg was first dried in a porcelain crucible and then ashed in an electric furnace. The drying was begun at a temperature of 40°C. which was held for 12 hours. During the next twelve hours the temperature was increased gradually to 100°C. By this time the yolk was dry, hard and dark brown. It was then removed to the muffle furnace and left at the lowest temperature for about 12 hours, after which the heat was increased every hour until a temperature of 1800°F. was reached. It was left at this temperature for 2 hours when the current was turned off. As soon as the crucibles had cooled sufficiently, they were removed to a desiccator and when cool, weighed. Forty cc. of 10 percent hydrochloric acid was added to the ash in the crucible and evaporated. Two successive forty cc. portions were added and brought to the boiling point then transferred to a volumetric flask, brought up to a volume of 100 cc. and transferred to a bottle until such time as the analyses could be made. Copper was determined by the Biazzo (17) method as modified by Elvehjem and Lindow (18). Iron was determined by the method of Fowweather (19). Calculations were made on undried egg yolk.

RESULTS

Two hundred and twelve egg yolks were analyzed for iron and copper and 208 egg yolks, in addition, were analyzed for iron, making in all 420 egg yolks analyzed for iron. The detailed results of the analyses are given in tables that are on file at the Library of the Experiment Station of the University of Kentucky. Averages and differences of averages are shown in Tables 1 and 2 and Figure 1.

Figure 1 shows graphically the average per pen of the iron and copper contents of egg yolk, calculated as mg. per 100 g. of yolk, per total yolk and as percentage of ash. The values upon which the chart is based are given in Table 1. Statistical analyses, given in Table 2, were made on the values calculated as mg. percent to note whether the differences between pens are significant.

Iron. Reference to Figure 1, shows that values calculated as mg. of iron per yolk do not always vary directly as the values calculated per 100 g. of yolk, which would seem to indicate some tendency for size of yolk to compensate for variation in percentage of iron content. For instance, if the average value for pen 6 per whole yolk is 1.14 mg. and that for pen 5 is 1.18 mg., while the mg. percentage value is 7.37 for pen 6 and 7.21 for pen 5, the discrepancy in the trend of the curve seems to be due to the difference in the average size of the yolks. This may also account for the difference in the trend of the curve when the values are computed as percentage of ash. However, the differences here may also be due to differences in other ash constituents that may be the result of the variations in treatment of hens.

In each of the three pairs of pens, the value of iron in the pen which received cod liver oil is higher than that in the one which did not receive oil with one exception, namely, when the values are calculated per total yolk, the average in pen 6 is slightly lower than in pen 5. It is not surprising, therefore, to find that in Table 2 statistical analysis shows that the difference between pens 5 and 6 is not significant, that the difference between pens 1 and 2 is real tho small and that the difference between pens 3 and 4 has a slight possibility of being significant.

Where pens 1, 3 and 5 are compared in Figure 1 with pens 2, 4 and 6, it will be noted that when the iron values are computed per 100 g. of yolk or as percentage of ash, there seems to be a tendency for sunshine and bluegrass to cause an increase in iron averages in the pens that do not receive cod liver oil but a decrease in the pens that receive oil.

Direct sunshine by itself does not increase the iron value as much as does cod liver oil by itself. (Compare pens 3 and 1 and pens 2 and 1 in Figure 1.) Neither do the two together, that is cod liver oil plus sunshine, have the effect that cod liver oil alone has. (Compare pens 4 and 1 and pens 2 and 1.) The same is true of direct sunshine plus bluegrass plus cod liver oil. The three together, pen 6, do not have the effect that cod liver oil alone has, pen 2. Nevertheless, it cannot be said that direct sunshine has no effect, because pens 3 and 5, neither of which received cod liver oil, show higher values than pen 1, which received neither cod liver oil nor direct sunshine. The fact that pen 5 is not appreciably higher than pen 3, and that pen 6 is lower even than pen 4 seems to indicate that the effect of bluegrass is probably negligible. Not only does the sunshine seem not to increase the effect of the cod liver oil but it might almost seem to nullify it when consideration is given to the fact that the drop in iron values from pen 2 to pen 4 is shown to be statistically significant in Table 2 as is also, of course, that between pens 2 and 6, since the value for pen 6 is less than that for pen 4.

Copper. Figure 1 indicates that, for each pair of pens, the one receiving cod liver oil has a higher average copper content in egg yolk than the one without oil. It is interesting to note also that direct sunshine alone seems to increase the average copper content above that of the pen without sunshine, and that direct sunshine plus bluegrass range seems to increase it still more, both in the pens that get cod liver oil and in the ones that do not. The values in copper steadily rise from the average in pen 1 to the average in pen 6. For instance, Table 1 shows that the value for pen 6 is practically two and one-half times that for pen 1. Both the bluegrass range and the direct sunshine seem to have an additive effect to that of cod liver oil in increasing

the copper content of egg yolk. From Table 2, it is seen that the differences between pens 1 and 2 and between pens 5 and 6 are statistically significant but not the difference between pens 3 and 4. The difference between pens 2 and 4 is not significant but between pens 2 and 6 and between pens 4 and 6 the differences are definitely significant.

The increase in iron and copper content that followed giving hens cod liver oil cannot be explained by the presence of these metals in the oil. The cod liver oil was analyzed for both copper and iron. Fifteen grams were taken for analysis and for neither of the metals was a positive test obtained with the amount used. Since the pens that received cod liver oil were given two percent in the basal ration and since it was estimated that each hen might consume 95 to 100 grams of feed per day, it is apparent that the quantity of iron and copper in the oil was too small, if present, to affect the values in the egg yolks. Even if one of these metals were present in amount sufficient for analysis, it is doubtful if it could affect the level in the eggs, in view of the results obtained by Elvehjem and co-workers (10) and Cunningham (11) referred to previously. This does not, of course, preclude the possibility of a catalytic effect of traces, occurring in amounts too small to detect by the methods used.

The fact that the copper content of the egg yolk is affected as much by sunshine as it is by cod liver oil, noted by comparing pens 1 and 2 and pens 1 and 3, points, also, to some other factor responsible for the differences rather than the copper content of the cod liver oil. When the hens are given cod liver oil and sunshine, the effect is greater than when given either one alone. The additional effect of open bluegrass range indicates that an energy factor may be responsible and may be found stored or held in cod liver oil and in bluegrass.

Cunningham (11) added small amounts of iron and copper to the diet of rats to note whether a storage of either or both would occur in the liver. His results showed a fairly high storage of iron in the liver of the rats when the iron content of the diet was increased. No evidence was found that feeding copper increased the absorption or storage of iron. The indications

were, as a matter of fact, that the amount of iron in the liver had decreased and that of the body had increased. Cunningham suggests the interpretation that the copper had promoted the utilization by the body of the stored iron in the liver or that possibly the presence of copper was necessary in changing inorganic iron to organic iron.

The progressively increasing copper content of egg yolks from pens 2, 4 and 6 as contrasted with the progressively decreasing values for iron in the yolks from the same pens may indicate that the factor (or factors) responsible for the storage of copper in the egg acts to promote greater utilization of iron by the hen and, therefore, less storage in the egg.

The problem of tissue respiration seems to be related to both copper and iron but the complexity of the interrelationships is such that no clear picture presents itself of the action involved. Warburg and Sakuma (20) have shown that iron is necessary for oxidation of thiol compounds, and Voegtlin, Johnson and Rosenthal (21) consider copper essential for the oxidation of glutathione. It is conceivable that copper may act as the catalyst for the oxidation of glutathione which in turn may act as an intermediary in the transfer of oxygen from oxyhemoglobin of the red cells to other tissues of the body and that the cysteine-cystine auto-oxidizable system aids in the reaction of oxygen with organic substances. That some wave length of energy in the sun's rays is capable of stimulating such catalytic action of copper and iron, does not seem impossible when the sensitivity of iron and copper-containing pigments to light is taken into consideration. The relatively high amounts of copper found in the skin and hair of animals, as reported by Lindow and co-workers (22) and Elvehjem and co-workers (10) may indicate that in its role as catalyst it needs activation by the sun's rays.

Since both sunshine and cod liver oil have an anti-rachitic effect, it may be that the factor involved is solely vitamin D. It is a well-known fact that deprivation of any of the vitamins leads to depressed bodily functions and that, as the deficiencies are made up, improvement of functioning and well-being results.

Hens deprived of vitamin D may have metabolism of copper and iron so retarded that storage in the egg is necessarily diminished. This seems to be borne out by the observation that the lowest iron and copper values of eggs produced are found in pen 1. Then, when either cod liver oil or sunshine or bluegrass is administered, all the bodily processes of the hen, including, of course, ovulation, may be so improved that not only are more eggs laid but the nutritive value of the eggs is increased, at least as regards their iron and copper content. If this is true, it is difficult to explain why the eggs from pens that received both direct sunshine and cod liver oil had a lower iron content than those that received cod liver oil alone, and why those from pens that received cod liver oil and were allowed open bluegrass range had a still lower iron content.

Because of the results obtained in this study, further work is being undertaken on the hemoglobin of the blood and on the copper and iron content of the blood and liver of the hens kept under the conditions of this experiment. Investigation will also be made on the glutathione and on the cysteine-cystine system of the blood and liver of the hens and on the egg yolks produced by them, in order to attempt to clarify the problem to some extent.

SUMMARY AND CONCLUSIONS

1. Investigation was made of the iron and copper content of the yolk of eggs produced by hens from six different pens, the differences in treatment being the administration of cod liver oil, sunshine and bluegrass range.

2. Addition of two percent of cod liver oil to the basal ration of hens raised the percentage level of both copper and iron values in the egg yolk.

3. Sunshine admitted directly to hens that did not have cod liver oil raised the percentage levels of copper and iron values in egg yolk over those of hens that received sunlight only thru ordinary window glass.

4. Sunshine admitted directly to hens that were given cod liver oil decreased the level of the iron but raised the level of the copper content of egg yolk over the eggs of hens that received sunshine only thru ordinary window glass. The effect of sunshine and cod liver oil on copper content seemed to be cumulative but on iron content the two seemed to have antagonistic effects.

5. Eggs produced by hens that received cod liver oil only, show higher percentage values of iron in the yolk than those produced by hens that received both cod liver oil and sunshine, and these in turn show higher values than those from hens allowed open bluegrass range and cod liver oil.

6. Hens allowed sunshine, bluegrass range and cod liver oil produced eggs having a higher copper content in the yolk than those from any other pen. The value was two and a half times as great as that of eggs produced in the pen confined without cod liver oil or direct sunshine or bluegrass.

TABLE 1. IRON AND COPPER CONTENT OF THE YOLK OF FRESH EGGS

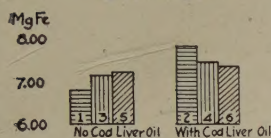
Pen	Treatments	No. Obs.*		Av. mg. in yolk		Av. % in ash		Av. mg. per 100 g. yolk	
		Fe	Cu	Fe	Cu	Fe	Cu	Fe	Cu
1	Confined, no cod liver oil.....	38	21	1.11	.126	.445	.050	6.82±.154	0.772±.047
2	Confined, 2% cod liver oil.....	66	35	1.24	.172	.487	.068	7.85±.108	1.096±.075
3	Sunshine, no cod liver oil.....	67	37	1.23	.189	.440	.067	7.16±.111	1.097±.079
4	Sunshine, 2% cod liver oil.....	57	25	1.25	.194	.465	.071	7.45±.111	1.142±.063
5	Bluegrass range, no cod liver oil	94	47	1.18	.209	.438	.081	7.21±.084	1.326±.082
6	Bluegrass range, 2% cod liver oil	99	47	1.14	.293	.451	.117	7.37±.083	1.927±.097

* Number of observations is the same as the number of eggs collected.

TABLE 2. DIFFERENCE OF THE MEANS IN MG. OF IRON AND COPPER PER 100 G. OF YOLK

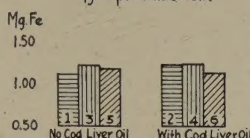
Pens	Variable	Fe		Cu	
		Difference	D	Difference	D
			P. E. diff.		P. E. diff.
1 & 2	Cod liver oil.....	1.02±.188	5.4	.324±.088	3.7
3 & 4	Cod liver oil.....	0.29±.157	1.8	.045±.101	0.4
5 & 6	Cod liver oil.....	0.16±.118	1.4	.601±.127	4.7
1 & 3	Sunshine	0.34±.190	1.8	.325±.092	3.5
2 & 4	Sunshine	0.40±.155	2.6	.046±.097	0.5
4 & 6	Bluegrass	0.03±.138	0.6	.785±.116	6.8
3 & 5	Bluegrass	0.05±.139	0.4	.229±.114	2.0
1 & 5	Sunshine and bluegrass.....	0.39±.176	2.2	.554±.094	5.9
2 & 6	Sunshine and bluegrass.....	0.48±.138	3.5	.831±.122	6.8

Mg:Fe per 100g. Yolk

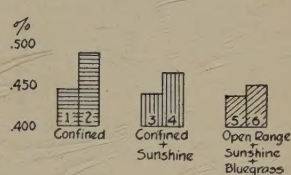
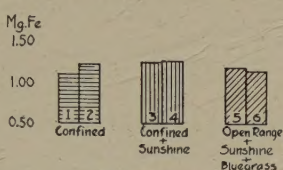
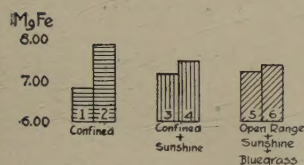
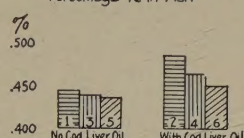


IRON

Mg:Fe per Whole Yolk

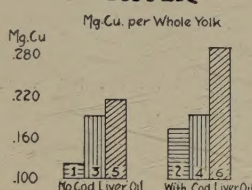
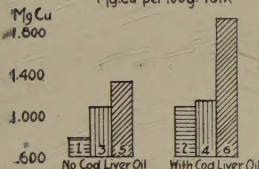


Percentage Fe in Ash

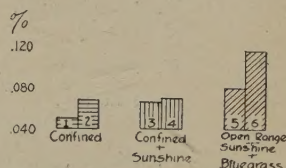
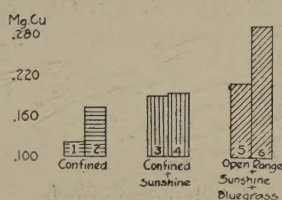
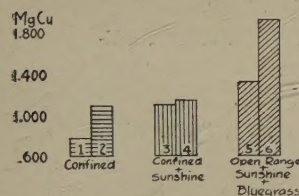
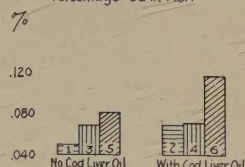


COPPER

Mg:Cu per 100g. Yolk



Percentage Cu in Ash



Pen 1. Confined, sunshine thru ordinary window glass, no cod liver oil

Pen 2. Confined, sunshine thru ordinary window glass, 2 percent cod liver oil.

Pen 3. Confined, sunshine on wire-screened sun porch, no cod liver oil.

Pen 4. Confined, sunshine on wire-screened sun porch, 2 percent cod liver oil.

Pen 5. Open bluegrass range, no cod liver oil.

Pen 6. Open bluegrass range, 2 percent cod liver oil.

REFERENCES

1. Robscheit-Robbins, F. S., *Physiol. Rev.*, 9, 666, 1929.
2. Whipple, G. H. and Robscheit-Robbins, F. S., *Amer. Jour. Physiol.*, 72, 395, 1925.
3. Gibson, R. B. and Howard, C. P., *Arch. Int. Med.*, 32, 1, 1923.
4. Miller, R. C. and Forbes, E. B., *Jour. of Nutrition*, 4, 483, 1931.
5. Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *Jour. Biol. Chem.*, 77, 797, 1928.
6. McHargue, J. S., Healy, D. J., and Hill, E. S., *Jour. Biol. Chem.*, 78, 637, 1928.
7. Payne, L. F., *Jour. Amer. Assn. Inst. and Invest. in Poultry Husbandry*, 6, 9, 1919.
8. Riddle, Oscar, *Amer. Jour. of Physiol.*, 94, 535, 1930.
9. Sherman, Henry C., *Chemistry of Food and Nutrition*, New York, 1932, 4th Edition, p. 327.
10. Elvehjem, C. A., Kemmerer, A. R., Hart, E. B. and Halpin, J. G., *Jour. Biol. Chem.*, 85, 89, 1929.
11. Cunningham, I. J., *Biochem. Jour.*, 25, 1267, 1931.
12. Peterson, W. H., and Elvehjem, C. A., *Jour. Biol. Chem.*, 78, 215, 1928.
13. Fleurent, E. and Levi, L., *Bull. Soc. Chim.*, 27, 441, 1920.
14. Guerithault, B., *These Pharm. Sup.*, Paris, 1927, Vigot freres, editeurs.
15. McHargue, J. S., *Amer. Jour. of Physiol.*, 72, 583, 1925.
16. Lindow, C. W., Elvehjem, C. A. and Peterson, W. H., *Jour. Biol. Chem.*, 82, 465, 1929.
17. Biazzo, R., *Ann. Chem. appl.*, 16, 2, 1926.
18. Elvehjem, C. A. and Lindow, C. W., *Jour. Biol. Chem.*, 81, 435, 1929.
19. Fowweather, F. S., *Biochem. J.*, 20, 93, 1926.
20. Warburg, Otto, and Sakuma, S., *Pflüger's Archiv.*, 200, 203, 1923.
21. Voegtlin, Carl, Johnson, J. M., and Rosenthal, S. M., *Jour. Biol. Chem.*, 93, 435, 1931.
22. Lindow, C. W., Peterson, W. H., and Steenbock, H., *Jour. Biol. Chem.*, 84, 419, 1929.